IS THE POTASSIUM CHANNEL DISTRIBUTION IN GLIAL CELLS OPTIMAL FOR SPATIAL BUFFERING OF POTASSIUM?

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ABSTRACT Glial cells in the nervous system are believed to reduce changes of extracellular potassium concentration ($[K^+]_o$), caused by neural activity, by carrying out spatial buffering of potassium. In the case of retinal glial cells (Müller cells), light-evoked increases of $[K^+]_o$ within the retina are reduced by K ions flowing through the Müller cell to the vitreous fluid of the eye. We have calculated the optimal way to distribute the potassium conductance of the Müller cell to maximize spatial buffering to the vitreous fluid. The best distribution is with half the potassium conductance in the outer part of the cell, where K^+ enters, and half the conductance in the vitreal endfoot, where K^+ leaves the cell. This calculated distribution is very different from the actual distribution measured by Newman (1984, *Nature* [*Lond.*], 309:155–157), where only 6% of the Müller cell conductance is in the outer cell and 94% is in the endfoot. The experimentally observed distribution gives less than a quarter of the spatial buffering that would be produced by the optimal distribution. The possible advantages of this arrangement are discussed.

INTRODUCTION

The extracellular potassium concentration, $[K^+]_o$, in the central nervous system must be maintained within certain limits if the brain is to function normally. Glial cells are believed to play an important role in controlling $[K^+]_o$ by carrying out K^+ spatial buffering (Orkand et al., 1966; Trachtenberg and Pollen, 1970; Gardner-Medwin, 1981, 1983): a localized rise in $[K^+]_o$ causes potassium to flow into glial cells and thus to be redistributed to areas where $[K^+]_o$ has not risen.

Recently, Newman (1984) has shown that the spatial distribution of potassium channels in isolated Müller cells, a type of retinal glial cell, is strikingly asymmetric. Although the cylindrical Müller cell stretches right through the retina from the photoreceptors to the vitreous fluid, 94% of its potassium conductance lies in the vitreal endfoot of the cell. The remaining 6% of the potassium conductance is in the parts of the Müller cell membrane facing the retinal neurons, where light-induced changes of $[K^+]_o$ occur (Kline et al., 1978; Steinberg et al., 1980). This confirmed previous evidence for such an asymmetry from intact retinas (Newman, 1980; Fujimoto and Tomita, 1981). Newman (1984) proposed that this asymmetric distribution of potassium channels is a key component of the Müller cell K^+ spatial buffering system. Rises of $[K^+]_o$ within the retina cause potassium to flow into the Müller cells, but 94% of this potassium will leave the cell, via the endfoot, into the vitreous fluid, which will function as a sink for the excess K^+. Since astrocytes in the central nervous system also possess endfeet near capillaries and the brain surface, Newman (1984) also suggested that K^+ spatial buffering by these cells would also be enhanced if their K^+ channel distribution were asymmetric, with most of the potassium conductance in the endfeet.

By analyzing a simple model of the Müller cell, we assess what distribution of potassium channels is optimal for spatial buffering of $[K^+]_o$ changes produced by neural activity in the retina.

THEORY

Our analysis is presented in two parts. First, we assume the electrical space constant of the cell to be much greater than the cell length so the cell can be treated as isopotential. This simplifies the mathematics and facilitates understanding of the results obtained. In the second section, we allow the membrane conductance to be arbitrarily high, so the space constant can be comparable to the cell length.

Electrical Space Constant Much Longer Than Cell

We consider a simplified model of the Müller cell (Fig. 1 *A*), in which the cell is divided into two parts: (*a*) the outer cell, within the retina, which is assumed to contain a fraction f of the total potassium conductance, G_K, and to be surrounded by a (variable) extracellular potassium concentration, K_o; (*b*) the vitreal endfoot, which is assumed to have a fraction $1 - f$ of the total potassium conductance, and to be surrounded by a (constant) extracellular potassium concentration, K_{ef}. The total potassium conductance, G_K, is assumed to be fixed: for example, the cell's biochemistry may only be able to sustain production of a fixed number of K^+ channels, the

optimal spatial distribution of which we wish to calculate. Membrane conductance to ions other than potassium is initially ignored (see below), as is active transport of K^+ across the cell membrane. The potassium concentration inside the cell, K_i, is assumed to be constant and position independent, so that the Nernst potential for K^+ is, in general, different at the two ends of the cell: $V_{K_o} = RT/F \log_e(K_o/K_i)$ at the outer cell, and $V_{K_{ef}} = RT/F \log_e(K_{ef}/K_i)$ at the endfoot.

We assume the membrane potential to be uniform in the cell. This is a reasonable assumption: considering the outer part of the cell, which Newman (1984) found to have a membrane resistance of 140 MΩ, to be a cylinder of length 80 μm and average diameter 9 μm filled with cytoplasm of resistivity 2 Ω m, we calculate a longitudinal electrical space constant of λ = 597 μm, i.e., much longer than the cell. For simplicity here, we have neglected the extracellular resistance, which would reduce λ slightly. We also ignore the small amount (< 7%) of voltage nonuniformity resulting from the outer part of the cell being terminated by the low-resistance endfoot. Including these complications would not significantly alter our conclusions, as is shown by the general analysis in the next section.

With these assumptions, the total current through the cell membrane is

$$fG_K(V - V_{K_o}) + (1 - f)G_K(V - V_{K_{ef}}), \quad (1)$$

and equating this to zero gives the resting potential as

$$V = fV_{K_o} + (1 - f)V_{K_{ef}}. \quad (2)$$

In the absence of potassium accumulation or depletion in the retina, V_{K_o} will equal $V_{K_{ef}}$, and this will also be the cell's resting potential. Consequently, no current will flow through the outer part of the Müller cell membrane. When K_o rises above K_{ef}, there will be a flux of K^+ in across the outer part of the Müller cell membrane, and an equal flux out of the endfoot into the vitreous fluid. The magnitude of this flux is given by

$$I_K = -fG_K(V - V_{K_o}), \quad (3)$$

or, using Eq. 2:

$$I_K = f(1 - f)G_K(V_{K_o} - V_{K_{ef}}). \quad (4)$$

This is the current responsible for spatial buffering of potassium toward the vitreous. It is proportional to the total potassium conductance of the cell (G_K). As is intuitively obvious, there is no spatial buffering however ($I_K = 0$), if the fraction f of potassium conductance at the membrane where $[K^+]_o$ rises is zero, because there is no route for K^+ into the cell. There is also no spatial buffering to the vitreous if $f = 1$, because then there are no channels in the endfoot to allow K^+ to leave the cell.

The dependence of I_K on f in Eq. 4 is plotted in Fig. 1 *B*. The maximum current, and maximum spatial buffering, occurs for $f = 1/2$, i.e., when 50% of the potassium conductance is located at the part of the cell where $[K^+]_o$ changes occur (outer cell) and 50% is located at the part of the cell where the extracellular potassium concentration is constant (endfoot). When $f = 0.06$, as found experimentally (Newman, 1984) the spatial buffer current is only 23% of what it would be with the optimal distribution of channels.

Calculations were also carried out assuming that the dependence of the membrane potassium currents on voltage and potassium concentration obeyed the Goldman equation (Goldman, 1943), rather than the ohmic relation of Eq. 1. These also predicted the optimal distribution of potassium channels for spatial buffering of small changes of $[K^+]_o$ to be with 50% of the potassium permeability in the outer part of the Müller cell, and 50% in the endfoot. As in Eq. 4, the spatial buffering current is approximately proportional to $f(1 - f)$, so for $f = 0.06$, the current is only 23% of the maximum possible value. Thus, the choice (above) of an ohmic dependence of I_K on V and V_K is not a particularly important assumption.

Recent work has suggested that glial cell membranes may not be perfectly K^+ specific (Chiu et al., 1984; Gray et al., 1984). To assess the importance of this for our calculated optimal distribution of K^+ conductance, we repeated the calculations presented above, assuming that the cell possessed a nonpotassium conductance (labeled sodium for convenience), G_{Na}, in addition to the potassium conductance, G_K. On this model, the total current through the cell membrane is

$$fG_K(V - V_{K_o}) + (1 - f)G_K(V - V_{K_{ef}}) + G_{Na}(V - V_{Na}).$$

By equating this to zero to calcuate the resting potential, we find that the change in potassium current across the outer part of the Müller cell membrane [i.e., the change in $fG_K(V - V_K)$] when the potassium concentration rises from K_{ef} to K_o is

$$\Delta I_K = \frac{f(1 - f + G_{Na}/G_K)G_K(V_{K_o} - V_{K_{ef}})}{1 + G_{Na}/G_K}. \quad (5)$$

This is plotted in Fig. 1 *B* for several values of G_{Na}/G_K.

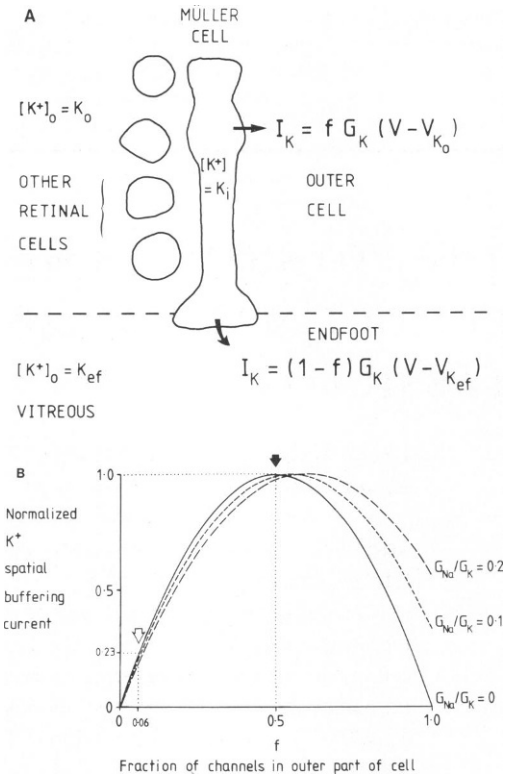

FIGURE 1 (*A*) Two-compartment model of Müller cell for the case where the electrical space constant is much longer than the cell. A fraction, f, of the total potassium conductance, G_K, is situated in the outer part of the cell, above the dotted line and within the retina. Below the dotted line, where the membrane of the endfoot is apposed to the vitreous humor, is the remaining fraction, $1 - f$, of the total G_K. The potassium concentration in the vitreous around the endfoot, K_{ef}, is constant, while K_o, the potassium concentration around the outer cell, is altered by neuronal activity. The internal potassium concentration, K_i, is assumed to be constant. The membrane potential is denoted V. The outward potassium current, I_K, through the endfoot, is the spatial buffering current. (*B*) Normalized spatial buffering current plotted against f for the model of part *A*. The solid line plots Eq. 4 for the case of a potassium-specific membrane ($G_{Na}/G_K = 0$). The clear arrow shows the value of f (0.06) found experimentally by Newman (1984). The black arrow shows the value of f (0.5) at which I_K would be a maximum. The dotted lines plot Eq. 5 for the case in which there is some conductance to ions other than K^+ (labeled G_{Na} for convenience).

Again, buffering of K^+ by the Müller cell does not occur ($\Delta I_K = 0$) if $f = 0$, i.e., there are no channels in the outer part of the cell through which K^+ can enter the cell. However, unlike the situation when the cell membrane is K^+ specific, buffering does occur when all the channels are in the outer part of the cell and none in the endfoot ($f = 1$). This is because a change in the K^+ current across the outer cell membrane can be balanced by a change in the nonpotassium current across the cell membrane: there is no need for an equal change in K^+ current across the endfoot membrane. (Of course, any K^+ entering the outer part of the cell that is not balanced by a corresponding efflux through the endfoot will accumulate intracellularly, and this type of buffering cannot be sustained indefinitely. If all the observed Müller cell potassium conductance were in the outer cell and $G_{Na}/G_K = 0.1$, one can calculate that the intracellular $[K^+]$ would rise at a rate of 0.37 mM/s if $[K^+]_o$ doubled.) The optimal fraction of potassium conductance to have in the outer part of the cell (to maximize Eq. 5) is $f = (1 + G_{Na}/G_K)/2$, i.e., greater than the value of 1/2 for a K^+-specific membrane. Thus, including nonpotassium channels in the Müller cell membrane shifts the optimal channel distribution even further from that observed experimentally.

So far we have assumed that the part of the Müller cell experiencing $[K^+]_o$ changes is all exposed to the same amount of K^+ accumulation (or depletion). This is only an approximation to the real situation, where, although about three-quarters of the Müller cell experiences an increased $[K^+]_o$ released from bipolar and amacrine cells when light is applied, the end of the Müller cell near the photoreceptors experiences a decrease in $[K^+]_o$ as potassium enters the receptors during illumination (Kline et al., 1978; Oakley et al., 1979; Steinberg et al., 1980). A generalization of the argument leading to Eq. 4 shows that, for a K^+-specific membrane, even with an arbitrary spatial profile, $V_{K_o}(x)$, for the potassium equilibrium potential around the outer part of the cell, the dependence on f of the spatial buffer current passing to the endfoot is as given by Eq. 4. Suppose the total potassium conductance in the outer cell is fG_K, distributed according to an arbitrary spatial profile $fG_K\,\rho\,(x)$ per unit length, where x is the distance from the outer end of the cell and $\int \rho\,(x)\,dx = 1$ (where the integration is done along the outer cell). The total current across the cell membrane is:

$$\int fG_K\,\rho(x)[V - V_{K_o}(x)]dx + (1 - f)G_K(V - V_{K_{ef}}),$$

giving a resting potential

$$V = f\int \rho\,(x)V_{K_o}(x)dx + (1 - f)V_{K_{ef}}.$$

The spatial buffer current to the endfoot is then:

$$I_K = (1 - f)G_K(V - V_{K_{ef}})$$

$$= f(1 - f)G_K\int \rho(x)\,[V_{K_o}(x) - V_{K_{ef}}]dx.$$

As in the case of a uniform $[K^+]_o$ change, to maximize K^+ transfer to and from the vitreous, the optimal fraction of channels to have in the outer cell is $f = 0.5$.

Electrical Space Constant Arbitrary

Eq. 4 indicates that, for a given distribution of potassium conductance (i.e., a given value of f), the spatial buffering current is increased if the total potassium conductance in the cell membrane is increased. However, if the membrane conductance becomes too high, our assumption that the electrical space constant is much greater than the cell length will become invalid. Indeed, if the potassium conductance found experimentally in Müller cells were redistributed so that 50% of it were in the outer cell, then the space constant of the outer cell would be reduced from 597 (calculated above) to 207 μ m. This may not be sufficiently greater than the cell length (80 μ m) for the assumption of isopotentially used in the preceding analysis to be valid.

In this section, we investigate the effect of the space constant being comparable to the cell length. (This analysis also includes the voltage nonuniformity resulting from termination of the outer cell by the low-resistance endfoot, which was neglected in the preceding section.) We assume the cell membrane conductance to be potassium-specific. The outer cell is treated (Fig. 2 *A*) as a uniform cable of length L and space constant λ, where λ is related to the fraction, f, in the outer cell of the total potassium conductance, G_K, by $\lambda^2 = L/(fG_K r_i)$, where r_i is the internal resistance per unit length. The cable equation for this part of the cell is

$$d^2(V - V_{K_o})/dx^2 = (V - V_{K_o})/\lambda^2. \qquad (6)$$

With the assumptions that no current leaves the end of the cell distant from the endfoot, and that the endfoot is represented as a region of resistance $R = 1/[(1 - f)G_K]$, through which a current $[V(x = L) - V_{K_{ef}}]/R$ flows, the solution of Eq. 6 is

$$V = V_{K_o} - \frac{(V_{K_o} - V_{K_{ef}})(e^{x/\lambda} + e^{-x/\lambda})}{e^{L/\lambda} + e^{-L/\lambda} + \frac{R}{r_i\lambda}(e^{L/\lambda} - e^{-L/\lambda})},$$

and the spatial buffer current flowing through the endfoot is

$$I_K = \frac{V_{K_o} - V_{K_{ef}}}{R + r_i\lambda\left(\frac{e^{2L/\lambda} + 1}{e^{2L/\lambda} - 1}\right)} \qquad (7)$$

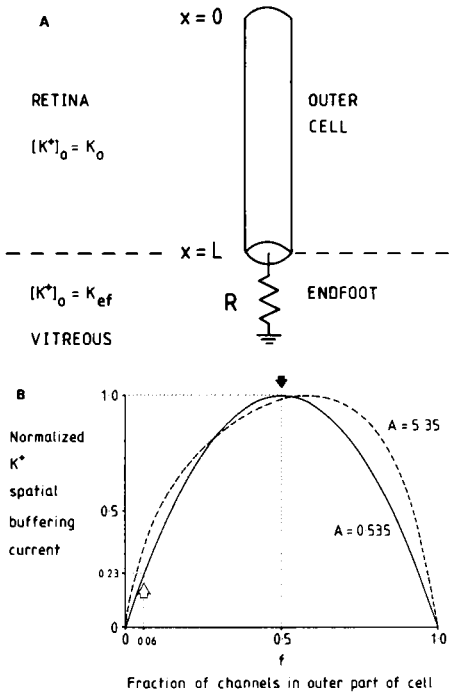

FIGURE 2 (*A*) Model of the Müller cell for the case where the electrical space constant is arbitrary. The outer part of the cell is considered as a uniform cable (of length L and electrical space constant λ) terminated by a resistance, R, representing the vitreal endfoot. K_{ef} and K_o have the same meaning as in Fig. 1. (*B*) Normalized spatial buffering current for the model of part *A*, plotted against f, the fraction of channels found in the outer part of the cell. The solid line plots Eq. 8 for the case when the parameter A ($= \sqrt{r_i L G_K}$, see text) is set to 0.535, i.e., appropriate for the total potassium conductance found experimentally in Müller cells (Newman, 1984). The clear arrow shows the value of f (0.06) found by Newman. The black arrow shows the value of f (0.5) at which I_K would be at a maximum. The dotted line shows the case in which the total potassium conductance G_K is increased 100-fold from the experimental value so that $A = 5.35$.

or

$$I_K = \frac{f(1-f)G_K(V_{K_o} - V_{K_{el}})}{f + A(1-f)\sqrt{f}\left(\frac{e^{2A\sqrt{f}}+1}{e^{2A\sqrt{f}}-1}\right)}, \quad (8)$$

where $A = \sqrt{r_i L G_K}$. This equation reduces to Eq. 4 in the limit of small L. Taking the values (Newman, 1984) $L = 80\ \mu$m, $G_K = 1/(8.8\ \text{M}\Omega)$, and $r_i = 3.14 \times 10^{10}\ \Omega\ \text{m}^{-1}$ (based on an average cell diameter of 9 μm and an internal resistivity of 2 Ω m), we find $A = 0.535$. Eq. 8 is plotted as a function of f for this value of A in Fig. 2 *B* (solid line). The curve is, within the thickness of the line, identical to the plot of Eq. 4 in Fig. 1 *B*. Thus, for the total potassium conductance found in Müller cells, the reduction of the space constant that would be produced if channels were relocated from the endfoot to the outer cell is not sufficient to alter the conclusions of the analysis presented above. The optimal distribution of conductance is with 50% in the endfoot and 50% in the outer cell, and the experimentally observed distribution gives only 23% of the spatial buffer current that the optimal distribution would allow.

Fig. 2 *B* also shows a plot of Eq. 8 for the case when the total potassium conductance in the cell is increased 100 times so that $A = 5.35$. The optimal fraction of channels to have in the outer cell is then $f \simeq 0.6$, i.e., larger than the optimal fraction for the limiting case of low total potassium conductance.

DISCUSSION

Our theoretical analysis demonstrates that, if a glial cell has a fixed number of potassium channels with which to carry out spatial buffering of K^+ to the vitreous, this buffering is maximized if half the potassium conductance is located at the part of the cell where $[K^+]_o$ changes occur and half at the part of the cell where $[K^+]_o$ is constant. This need not imply equal numbers of potassium channels in these two regions: the properties of the channels (conductance or fraction of time open) could be different in the two parts of the cell. Furthermore, our theory need not imply that the potassium conductance per unit area of cell membrane should be the same in the two regions: the cell area exposed to $[K^+]_o$ changes may (as in the case of the Müller cell) be much larger than the area exposed to a constant $[K^+]_o$. Nevertheless, Figs. 1 and 2 show that the nonuniformity of potassium conductance density in Müller cells is so great as to reduce spatial buffering to only 23% of what it could be.

Why is the distribution of potassium conductance in Müller cells so far from being optimal for spatial buffering to the vitreous? One possibility is that the low potassium conductance in the outer part of the cell serves to reduce spatial buffering between different parts of the neural retina, for example from the inner retina where $[K^+]_o$ rises, to the photoreceptor layer where $[K^+]_o$ falls. (This may be important if the $[K^+]_o$ decrease around the photoreceptors serves some function.)

Another possibility involves lateral spatial buffering of potassium. Our analysis has considered only spatial buffering of potassium in the direction of the main axis of the Müller cell. This is appropriate for the case of uniform illumination of the retina, when the extracellular potassium concentration at a given retinal depth will be independent of lateral position in the retina. Normally, however, the retina will be nonuniformly illuminated, and lateral movement of K^+ must also be considered. By analogy with glial cells in other parts of the nervous system (Kuffler and Nicholls, 1966), we would expect neighboring Müller cells in the retina to be electrically coupled to each other. The observation of gap junctions between Müller cell processes, and the large receptive fields of these cells (Miller and Dowling, 1970; Gold and Dowling, 1979), support this idea (although it does not seem to have been clearly shown that the gap junctions are between Müller cells rather than different processes of the same cell). Thus, during localized illumination of the retina, K^+ accumulating under the region of illumination will be spatially buffered, not only to the vitreous, but also laterally through the nearby Müller cells (to an extent that depends on the number and conductance of the gap junctions linking the cells). This lateral spatial buffering will aid the reduction of $[K^+]_o$ in the illuminated region, but will increase $[K^+]_o$ in the surrounding retina. Consequently, bipolar, amacrine, and ganglion cells outside the illuminated region will be depolarized, and as a result the spatial resolution of the retina may be reduced. In fact, the "on" pathways in the retina (depolarizing bipolar and on ganglion cells) will have their spatial resolution reduced, while for the "off" pathways (hyperpolarizing bipolars and off ganglion cells), the depolarization induced by lateral buffering of K^+ will be a form of lateral inhibition similar to that produced by horizontal and amacrine cells.

We propose that the concentration of potassium conductance in the Müller cell endfoot serves to impose a preferred direction, toward the endfoot, on K^+ spatial buffering in order to avoid this possible degradation of spatial resolution; having the conductance in the outer cell lower than our optimal calculated distribution will reduce spatial buffering between the outer parts of neighboring Müller cells.

An alternative hypothesis is that spatial buffering of potassium from the retina to the vitreous is not the only function of the Müller cell potassium channels, and that other, as yet unknown, constraints require the endfoot conductance to be much higher than the rest of the cell. The observation of specialized regions of contact between endfoot processes and nearby ganglion cell axons (Hildebrand and Waxman, 1983) may suggest, for example, that mediation of spatial buffering within the plane of the endfoot is also an important Müller cell function.

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